REMARKS

The Invention

The invention provides a diagnostic method in which the degree of methylation of one or more C residues in CpG sequences within the promoter region and the 5' end of the coding sequence of the HIN-1 gene in a test cell is determined. A high degree of methylation at this site is an indication that the test cell is a cancer cell.

Status of the claims

Claims 23-46 are pending and claims 23 and 24 and 35-46 are under consideration in this application, claims 1-22 having been cancelled and claims 25-34 having been withdrawn from consideration as allegedly being drawn to separate inventions. All the claims under consideration stand rejected. After entry of the above amendments, claims 23-46 will be pending and claims 23, 24, and 35-46 will be under consideration.

Objection to Specification

The Examiner asserts that hyperlinks remain in the application and refers to text in the application still containing a hyperlink (Office Action, page 2, lines 12-16). Applicants submit that the hyperlink referred to by the Examiner is the hyperlink that Applicants intended to delete in the prior Response (submitted August 28, 2003) but, in the preamble to the amendment (see page 2, line 2, of the prior Response), inadvertently referred to the incorrect page of the application (page 4 rather than page 14). However, in the Remarks section of the prior Response (page 6, line 19), Applicants referred to the correct page of the application. Applicants repeat the deletion of the hyperlink herein and, based on further review of the application, believe that it contains no additional hyperlinks. Applicants apologize for the error.

35 U.S.C. § 112, second paragraph, rejections

(a) Maintained rejection

Claims 23-24 remain rejected and claims 44-46 are rejected as allegedly being vague and indefinite.

On page 3, lines 1-7, of the Office Action, the Examiner has reiterated his position first stated in the Office Action of March 31, 2003, that the "term HIN-1 in association with a promoter region" is vague and indefinite. In the Response filed August 28, 2003, Applicants provided an extensive argument as to why the term is not vague and indefinite. The Examiner has not addressed this argument but merely restates the assertion that the term is vague and indefinite and indicates that the rejection can be overcome by adding a sequence identifier. For the convenience of the Examiner, Applicants restate below the argument presented in the Response filed August 28, 2003.

> The specification discloses full-length cDNA sequences corresponding to human and mouse HIN-1 genes (SEQ ID NOs: 3 and 7, respectively; Figs. 1A and 3A) and all but a small part of the 5' end of leader sequence of a cDNA corresponding to the rat HIN-1 gene (SEQ ID NO: 20; Fig. 9A). The protein encoded by the human gene is 60.8% homologous to that encoded by the mouse gene and . . . 62% homologous to that encoded by the rat gene; the rat HIN-1 protein is 84% homologous to the mouse HIN-protein (see Example 2). The mouse and human HIN-1 proteins, at least, are the same length (i.e., 104 amino acids) and it seems likely that the rat protein has the same, or a very close, length (e.g., Example 2). From this information in the specification, the description of HIN-1 gene expression patterns in various normal and malignant tissues (e.g., Example 3), and the depiction of part of the human HIN-1 promoter region (SEQ ID NO:19; Fig. 8), one of skill in the art would readily be able to discriminate, or establish the identity between, a HIN-1 gene of the instant application, and any other gene hypothetically and coincidentally also designated HIN-1.

Applicants respectfully submit that, in view of these considerations, one of skill in the art could readily identify a HIN-1 promoter region and that there is no reason why the region of interest be limited by a particular sequence identifier. Requiring this would exclude from at least the literal scope of the claim assays performed on DNA containing even the most trivial allelic differences from an HIN-1 promoter region defined by a particular sequence identifier.

Notwithstanding the above considerations, in the interest of even greater clarity, Applicants have changed the term "HIN-1 promoter region" to "a CpG island in the HIN-1 5' promoter region". This amendment is supported by the specification (e.g., at page 26, lines 14-18) and adds no new matter.

(b) New rejections

(i) Claims 23-24 and 35-46 stand rejected as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter that Applicants regard as the invention.

From the comments on page 3, lines 9-18, of the Office Action, Applicants understand the Examiner's position to be that neither the claim nor the specification defines the relative term "a high degree" with respect to methylation of C residues in the HIN-1 promoter region. Applicants disagree with this position. For example, the description of Fig. 6 on page 8 of the specification provides a key to the semi-quantitative methylation data presented in Fig. 6 and obtained in the experiment described in Example 4. Applicants submit that, from this key, one of skill in the art would understand what is meant by a "high degree of methylation". Nevertheless, in order to expedite prosecution of the instant application, Applicants have (as suggested by the Examiner) added a step to claim 23 specifying a comparison between test and control data. This amendment is supported by the specification (e.g., at page 28, lines 3-15) and adds no new matter.

(ii) Claims 38-43 stand rejected on the grounds that the term "the segment" lacks antecedent basis.

The Examiner points out on page 4, lines 3-5, of the Office Action that the term "the segment" in the above-listed claims lacks antecedent basis. The rejection is rendered moot by the replacement in claims 38-43 of the term "the segment" with "the nucleotide sequence", which is recited in parent claim 23. For consistency, Applicants have similarly amended claim 45.

In light of the above considerations, Applicants respectfully request that the rejections under 35 U.S.C. § 112, second paragraph, be withdrawn.

35 U.S.C. § 112, first paragraph, rejections

(a) Claims 38-41 stand rejected on the grounds that the specification allegedly does not contain a written description of the claimed invention. Applicants respectfully traverse the rejection.

From the comments on page 4, line 12, to page 5, line 2, of the Office Action, Applicants understand the Examiner's position to be that the specification does not provide support for the terms "nucleotide 1 to nucleotide 252 of SEQ ID NO:19" and "nucleotide 229 to nucleotide 551 of SEQ ID NO:19". Applicants disagree with this position for the reasons given below.

On page 36, lines 30-31, of the specification (in Example 4), it is stated that the "-532 to +31" region analyzed in the experiment described in Example 4 (data from which is depicted in Fig. 6A) "contained the sequence shown in Fig. 8 (SEQ ID NO:19) and the first 12 nucleotides of the hHIN-1 coding sequence." From this statement, it is clear that nucleotide "-532" of the "-532 to +31" studied in the experiment corresponds to residue 1 of SEQ ID NO:19. Any concern that the word "contained" in the above-quoted statement could mean "included or "comprised" rather than "consisted of" is dispelled by the following considerations: (i) a DNA region defined as extending from nucleotide -532 to nucleotide +31 would be understood by one skilled in the art to be **563** nucleotides in length; and (ii) the total number of nucleotides in SEQ ID NO:19 (consisting of 551 nucleotides; see the Sequence Listing) plus the first twelve nucleotides of the hHIN-1 coding sequence (i.e., SEQ ID NO:3) is **563**.

In Example 4 a subregion ("-532 to -281") of the "-532 to +31" region was also subjected to methylation analysis (see page 36, line 29, of the specification). By reference to SEQ ID NO:19 in the Sequence Listing, it is clear that the "-532 to -281" subregion corresponds to the "nucleotide 1 to nucleotide 252 of SEQ ID NO:19" subregion specified by claims 38 and 39.

Another subregion of the "-532 to +31" region analyzed in the Experiments described in Example 4 was the "-304 to +31" subregion (see page 36, lines 28-30, of the specification). On page 37, lines 1-2, of the specification it is stated that the " '-304 to +31' region contained the 3'

323 nucleotides of SEQ ID NO19 and the first 12 nucleotides of the hHIN-1 coding sequence." From this statement and the Sequence Listing it is apparent that the "-304 to +31" region corresponds to "nucleotide 229 to nucleotide 551 of SEQ ID NO:19 and nucleotide 1 to nucleotide 12 of SEQ ID NO:3" as specified by claims 40 and 41. Applicants could have used language identical to that in the specification to define each of these same regions, but instead complied with U.S. Patent and Trademark Office policy requiring that sequences mentioned in claims always be described by sequence identifiers.

In view of the above considerations, Applicants submit that specification provides the required written description for the terms "nucleotide 1 to nucleotide 252 of SEQ ID NO:19" and "nucleotide 229 to nucleotide 551 of SEQ ID NO:19".

(b) Claims 23 and 35-46 stand rejected on the grounds that the specification allegedly does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with these claims. Applicants respectfully traverse the rejection.

From the comments on page 5, line 3, to page 8, line 15, of the Office Action, Applicants understand the Examiner to be asserting that it would require undue experimentation for one skilled in the art to determine: (1) what levels of methylation the terms "high" and "moderate" define; (2) for what cancers the method would be diagnostic, as the only control non-cancerous tissue in which HIN-1 promoter region methylation was measured was breast tissue; and (3) what "nucleotide regions" are covered by the term "HIN-1 promoter region".

Assertion (1) is moot in light of the above amendment to claim 23 adding a control cell comparison step.

With respect assertion (2), Applicants submit that one of skill in art would conclude that, for the reasons given below, the claimed method would be diagnostic for cancers in addition to breast cancer and, given the teaching of the specification, would be able without undue

experimentation to establish whether the claimed HIN-1 promoter region methylation assay is diagnostic for a cancer of interest.

Applicants observed that in a range of normal tissues (including lung, prostate, and pancreas in addition to breast) there is significant HIN-1 expression (see page 5, lines 6-9 of the specification). In addition, they observed that in primary lung cancers and a pancreatic cancer cell line there was, as in breast cancer, greatly reduced HIN-1 expression (see page 36, lines 3-6, and page 37, lines 24-25 of the specification). Applicants embarked on a series of experiments aimed at establishing the mechanism of reduced HIN-1 expression in cancer cells (see Example 4 in the specification). They discovered that in breast cancer cells there is a much higher level of HIN-1 promoter region methylation than in normal breast tissue (see page 36, line 23, to page 37, line 24, of the specification). Moreover, their experiments indicated that this methylation was at least one cause of the lower HIN-1 expression in the breast cancer cells (see page 38, lines 1-13, of the specification). Applicants submit, that in view of these findings, one of skill in the art would reasonably expect that in HIN-1-expressing normal tissues in general (e.g., normal lung, prostate, and pancreatic tissue) there would be, as in normal breast tissue, a lower level of HIN-1 promoter region methylation than in corresponding cancerous tissues.

Consistent with this expectation, work of some of the instant inventors, after the priority of the instant application, has shown that, while HIN-1 promoter regions in lung, prostate, and pancreatic cancers were significantly methylated, they were essentially unmethylated in lung and prostate samples from non-cancer patients [See pages 6-9 of a manuscript (Krop et al. *Frequent HIN-1 promoter methylation and lack of expression in multiple human tumor types*) enclosed as Exhibit A]. Interestingly, intermediate levels of methylation were seen in non-cancerous tissue immediately adjacent to cancerous tissue of prostate and pancreatic cancer patients (see page 7, line 19, to page 8, line 5, of Exhibit A). This phenomenon could have been due to cancer cells "contaminating" the non-cancerous tissue or to pre-malignant changes occurring in the non-cancerous tissue.

In addition, Wong et al. (Clin. Cancer Res. 9:3042-3046, 2003; copy enclosed as Exhibit B) have shown lack of HIN-1 promoter region methylation in a variety of normal cell types

including cells in adenoid-derived epithelial cell cultures, tonsil-derived epithelial cell cultures, skin-derived fibroblasts, normal adenoid tissue, and nasopharyngeal swabs, throat-rinsing fluid of healthy individuals, and peripheral blood from normal individuals. On the other hand, HIN-1 promoter region methylation was observed in five out of five nasopharyngeal carcinoma (NPC) cell lines, 36 out of 47 primary NPCs, and a significant number of nasopharyngeal swabs, throat-rinsing fluids, and peripheral blood samples from NPC patients (page 3043, column 1, paragraph 7, to page 3044, column 1, paragraph 2). Applicants submit that, in light of the above considerations, one of skill in the art would believe that assays for HIN-1 promoter region methylation are likely to be useful diagnostic tests for a broad spectrum of cancers.

With respect to assertion (3), the Examiner states that:

> "[c]learly it would be expected that a substantial number of the polynucleotide molecules encompassed by the claims **would not** share either structural or functions properties with HIN-1 promoter regions." (emphasis in original) (page 8, lines 10-12, of the Office Action)

Applicants do not understand this argument, as the polynucleotide molecules referred to by the Examiner are HIN-1 promoter regions. Moreover, in view of the arguments and amendments described above, HIN-1 promoter regions are more than adequately described by both claim 23 *per se* and the specification. Applicants respectfully submit that one of skill in the art, reading claim 23 and the parts of the specification referred to above, would be adequately apprised of the genomic region that can be subjected to methylation analysis in order to discriminate a cancer of a tissue of interest from corresponding non-cancerous tissue and thus would be able to develop, without undue experimentation, a workable cancer diagnostic assay within the scope of claim 23.

Applicants submit that it is implicit in claim 23 that the nucleic acid analyzed by the specified method is genomic DNA. However, in order to allay the Examiner's concern that "the promoter region clearly encompass (*sic*) a variety of species including full-length cDNAs, genes, and protein coding regions" (Office Action, page 8, lines 8-10), Applicants have amended claim 23 to specify that the nucleotide sequence analyzed by the claimed method be in a

"genomic segment". This amendment is supported by the specification (e.g., at page 30, line 29, to page 31, line 3) and adds no new matter.

In light of the above considerations, Applicants respectfully request that the rejections under 35 U.S.C. § 112, first paragraph, be withdrawn.

CONCLUSION

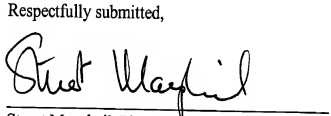
In summary, for the reasons set forth above, Applicants maintain that the pending claims patentably define the invention. Applicants request that the Examiner reconsider the rejections as set forth in the Office Action, and permit the pending claims to pass to allowance.

If the Examiner would like to discuss any of the issues raised in the Office Action, Applicants' undersigned representative can be reached at the telephone number listed above

Enclosed is a request for an automatic extension of time and a check in payment of the extension in time. Please apply any other charges or credits to Deposit Account No. 06-1050, referencing Attorney Docket No. 00530-094001.

Respectfully submitted,

Date: 3/15/04



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